HELIA, **24**, Nr. 35, p.p. 11-16, (2001) UDC 631.523:632.954:633.854.78

INHERITANCE OF IMIDAZOLINONE-HERBICIDE RESISTANCE IN SUNFLOWER

J. M. Bruniard[1] and J. F. Miller[2*]

[1]*A.C.A., Ruta 8, Km 232, 2700 Pergamino, Republica Argentina*
[2]*USDA-ARS, Northern Crop Science Laboratory, PO Box 5677, Fargo, ND, USA*

Received: September 14, 2001
Accepted: December 05, 2001

SUMMARY

Broadleaf weeds cause considerable yield losses to sunflower production in all regions of the world. Resistance to the imidazolinone herbicides, imazethapyr and imazamox, found in a population of wild sunflower, could have great value for controlling many broadleaf weeds. The herbicide resistance was successfully transferred from resistant wild sunflower plants to a cultivated sunflower inbred line, HA 425. The objective of this investigation was to determine the inheritance of resistance to the herbicide imazamox in HA 425. Segregation ratios of plants in F_2 and testcross populations indicated that resistance was controlled by two genes, a major gene having a semi-dominant type of gene action (*Imr1*), and a second gene (*Imr2*) with a modifier effect when the major gene is present. Resistance in sunflower can only be achieved with homozygocity (*Imr1, Imr1, Imr2, Imr2*) of both resistance genes in an inbred line or in a hybrid. Completely resistant hybrids require having resistance factors in both parents.

Key words: genetics, herbicide resistance, wild *Helianthus*, sunflower

INTRODUCTION

Hybrid sunflower (*Helianthus annuus* L.) producers have very few herbicides available for controlling broadleaf weeds. Resistance to the imidazolinone herbicides imazethapyr (Pursuit) and imazamox (Raptor) has been found in a population of wild sunflower (wild *H. annuus* L.), and it could have great potential for producers in all regions of the world. The two herbicides control several broadleaf weeds including *Kochia scoparia*, *Brassica arvensis*, *Amaranthus retroflexus*, *Solanum nigrum*, *Xanthium pennsylvanicum*, *Salsola kali*, *Iva zanthifolia*, *Polygonum coccineum*, *Ambrosia artemisiifolia*, *Polygonum convolvulus*, wild *Helianthus annuus* and *Chenopodium album* (Zollinger, 1997). In addition, these two herbi-

* Corresponding author

cides may control broomrape (*Orobanche cernua* Loefl.) in areas of the world where this parasitic weed infests sunflower (Alonso *et al.*, 1998).

The discovery (Al-Khatib *et al.*, 1998) of a wild sunflower population (wild *Helianthus annuus* L.) resistant to the imidazolinone and sulfonylurea classes of herbicides has caused excitement in the sunflower industry. The objective of this investigation was to determine the inheritance of resistance to the herbicide imazamox, with the resistance derived from wild plants from the original population.

MATERIALS AND METHODS

Wild sunflower seed samples of *Helianthus annuus* were collected from a soybean field near Rossville, Kansas, that had been treated with imazethapyr for seven consecutive years. Approximately 300 seeds of the wild population were subjected to the germination technique developed by Chandler and Jan (1985), utilizing scarification of seeds and 100 ppm gibberellic acid (GA_3). One hundred forty-four plants were obtained. At the V6 (Schneiter and Miller, 1981) plant stage (leaf 5 and 6 being approximately 6.5 cm in length), the plants were treated in a special spray chamber at North Dakota State University with imazethapyr dispersed in water at the 15X rate (11.25 ml l^{-1}), the labeled rate for soybean. The X77 surfactant was also used at a 2.5 ml l^{-1} rate.

Twelve of the original 140 plants tested were found to be extremely resistant to the 15X rate. Pollen from one resistant plant was collected and crossed with HA 89 (PI 599773). Approximately 10 to 12 days after pollination, embryos were collected and cultured (Chandler and Beard, 1983) to obtain plants. When the F_1 plants reached the V6 stage, plants were treated with imazamox (Raptor) at a 1X rate (3.0 ml l^{-1} or 35 g AI ha^{-1}). Resistant plants were identified and crossed to HA 89. The same backcrossing procedure utilizing imazamox as the screening herbicide was continued to obtain BC_2F_1 plants which were self-pollinated to produce BC_2F_2 seed. The pedigree breeding method was used to develop a BC_2F_6 line utilizing imazamox at a 1X rate (3.0 ml l^{-1}) in the greenhouse and a 2X rate (6.0 ml l^{-1}) in the field which was released by the USDA-ARS as HA 425.

The following populations were utilized in the inheritance investigation: parents (HA 425 and HA 89), the F_2 population of the cross HA 425/HA 89, and the testcross population HA425/HA89//HA 89. The F_1 plants of the cross HA 425/HA 89 were screened for resistance to imazamox by applying a 2X rate (6.0 ml l^{-1}) at the V6 stage. F_1 plants showed an intermediate reaction and were self-pollinated to produce the F_2 seed. One F_1 plant was randomly selected and emasculated. This plant was pollinated with the susceptible line HA 89 to produce seed for the testcross population.

A population of 118 F_2 plants was screened for resistance to imazamox in the greenhouse at the USDA Northern Crop Science Laboratory, and 137 plants were screened for resistance to imazamox in the field at Pergamino, Argentina. At the V6

stage, the plants were sprayed with imazamox at a 2X (6.0 ml l^{-1}) rate. After six days, symptoms of the imazamox application were clearly visible. Individual F_2 plants were phenotypically classified as follows: resistant, (those plants completely green with no symptoms); intermediate, (when the plant withstood the herbicide treatment but showed a definite degree of chlorosis symptom); and susceptible, (when plants died).

The same procedure was followed with the testcross population, consisting of 86 plants grown in the greenhouse. After seven days, individual testcross plants were phenotypically classified as follows: intermediate, when the plant withstood the herbicide treatment but showed diverse degrees of chlorosis symptom with two subclasses, intermediate plus (I+) and intermediate minus (I-); and susceptible, when plants showed extreme chlorosis (S+) or died after seven days (S-). Genotypic distributions of the F_2 and testcross populations were analyzed according to Chi-square tests for goodness of fit and heterogeneity at the 5% level.

RESULTS AND DISCUSSION

The genotype HA 425 was completely resistant and showed no symptoms of chlorosis in the greenhouse or in the field after treatment with imazamox. The susceptible genotype HA 89 died six days after treatment, with complete burning of the meristematic tissue. Three distinct phenotypic classes, resistant (R), intermediate (I), and susceptible (S), were identified in the F_2 populations following herbicide application. Based on the three classes, two different models were postulated. The first model tested a single, major gene having a semi-dominant type of gene action with the heterozygous genotype being intermediate in resistance. The second model tested a major gene having a semi-dominant type of gene action and a second gene with a modifier effect when the major gene is present. Three phenotypic classes would be expected in both models with the first model having an expected 1:2:1 resistant:intermediate:susceptible ratio and the second model having an expected 3:9:4 resistant:intermediate:susceptible ratio.

The Chi-square goodness of fit values for the F_2 populations fit both the 1:2:1 and the 3:9:4 ratios (Table 1). However, the χ^2 probability values of the 3:9:4 ratio for the populations and the deviation χ^2 were significantly lower than for the 1:2:1 ratio. In all populations observed, the number of resistant plants was always smaller than expected in the 1:2:1 ratio. Therefore, the second model was accepted.

In the testcross population, four distinct classes were observed: intermediate plus (I+), intermediate minus (I-), susceptible plus (S+), and susceptible minus (S-) (Table 2). These classes only fit the second model of a major gene having a semi-dominant type of gene action and a second gene with a modifier effect when the major gene is present. A single, major gene would segregate into a 1:1 ratio in the testcross population, intermediate and susceptible. The data for the testcross fit a 1:1:1:1 ratio, indicating control by two genes and the second model was accepted.

Table 1: Chi-square (χ^2) goodness of fit values for the F_2 populations screened for imazamox resistance

Populations	F_2 data							
	Number of plants				Ratio tested 1:2:1		Ratio tested 3:9:4	
	R †	I	S	Total	$\chi^{2\,\ddagger\ddagger}$	*P* ¶	$\chi^{2\,\ddagger\ddagger}$	*P* ¶
Greenhouse	23	63	32	118	1.91	0.25 - 0.50	0.41	0.75 -0.95
Field Argentina	24	74	39	137	4.17	0.10 - 0.25	0.89	0.50 - 0.75
Total	47	137	71	255				
Deviation $\chi^{2\,\#}$					5.93	0.01 - 0.05	1.12	0.50 - 0.75
Heterogeneity χ^2 ††					0.17	0.90 - 0.95	0.17	0.90 - 0.95

† R, I, and S = resistant, intermediate and susceptible, respectively
‡‡ df =2
¶ Probability
# Deviation chi-square, df = 2
†† Heterogeneity chi-square, df = 2

In order to confirm the hypothesis of the one semi-dominant and one modifier gene model, randomly selected F_2-derived F_3 plants within classes from both the F_2 and the testcross populations were progeny tested in Argentina under field conditions. Some of the progeny of the testcross populations segregated in a consistent manner, but some F_3 families derived from F_2 plants that were completely resistant definitely segregated in ratios not compatible with a one gene model. One intermediate type was also homozygous intermediate in the field, and F_4 progeny of this line did not segregate in tests at Fargo the next year. The fact that this plant did not segregate was not consistent with a one gene model.

Table 2: Chi-square (χ^2) goodness of fit values for the testcross population screened for imidazolinone resistance

Population	Testcross data						
	Number of plants					Ratio tested 1:1:1:1	
Sub class	I+ †	I-	S+	S-	Total	$\chi^{2\ddagger\ddagger}$	*P* ¶
Greenhouse	22	18	19	22	81	0.63	0.75 - 0.50

† I+, I-, S+, and S- = intermediate plus, intermediate minus, susceptible plus, and susceptible minus, respectively
‡‡ df = 2
¶ Probability

This study indicates that imazamox herbicide resistance in sunflower is controlled by one semi-dominant gene, designated *Imr1*, and a second modifier gene, designated *Imr2*. The genotype of HA 425 would be *Imr1, Imr1, Imr2, Imr2*. The genotype of the F_2-derived F_3 intermediate plant found in Argentina would be *Imr1, Imr1, imr2, imr2*. The genotype, phenotype, and phenotypic ratios for the F_2 and testcross populations are shown in Tables 3 and 4.

Table 3: Genotype, phenotype, and phenotypic ratio of sunflower-resistant ALS alleles for the model with one semi-dominant gene (*Imr1*) and a second modifier gene *(Imr2*) in F_2 populations

Genotype (Ratio)	Phenotype	Phenotypic ratio
Imr1, Imr1 Imr2, Imr2 (1)	Resistant	3
Imr1, Imr1 Imr2, imr2 (2)	Resistant	
Imr1, imr1 Imr2, Imr2 (2)	Intermediate	9
Imr1, imr1 Imr2, imr2 (4)	Intermediate	
Imr1, Imr1 imr2, imr2 (1)	Intermediate	
Imr1, imr1 imr2, imr2 (2)	Intermediate	
imr1, imr1 Imr2, Imr2 (1)	Susceptible	4
imr1, imr1 Imr2, imr2 (2)	Susceptible	
imr1, imr1 imr2, imr2 (1)	Susceptible	

Table 4: Genotype, phenotype, and phenotypic ratio of sunflower-resistant ALS alleles for the model with one semi-dominant gene (*Imr1*) and a second modifier gene (*Imr2*) in a testcross population

Genotype (Ratio)	Phenotype (subclasses)	Phenotypic ratio
Imr1, imr1 Imr2, imr2 (1)	Intermediate (+)	1
Imr1, imr1 imr2, imr2 (1)	Intermediate (-)	1
imr1 ,imr1 Imr2, imr2 (1)	Susceptible (+)	1
imr1, imr1 imr2, imr2 (1)	Susceptible (-)	1

The consequence of breeding for a character controlled by the two gene model of one major semi-dominant gene and a second modifier gene is that truly non-segregating resistance can only be achieved with complete homozygosity (*Imr1, Imr1, Imr2, Imr2*) in an inbred line or in a hybrid. Complete resistance in hybrids requires having resistance factors in both parents. In backcrossing the resistance trait to elite lines, the BCF_1 generation will be intermediate (*Imr1, imr1, Imr2, imr2*), and not have complete resistance. Therefore, care must be taken regarding the rate of herbicide used to screen these plants. One or more generations of self-pollination and progeny testing will be necessary to clarify the classification of phenotypes, similar to the situation when breeders work with a recessive trait.

REFERENCES

Al-Khatib, K., J.R. Baumgartner, D.E. Peterson and R.S. Currie, 1998. Imazethapyr resistance in common sunflower (*Helianthus annuus*). Weed Sci., 46: 403-407.

Alonso, L.C., M.I. Rodriguez-Ojeda, J. Fernandez-Escobar and G. Lopez-Ruiz-Calero, 1998. Chemical Control of broomrape (*Orobanche cernua* Loefl.) in sunflower (*Helianthus annuus* L.) resistant to imazethapyr herbicide. Helia, 21(29): 45-54.

Chandler, J.M. and B.H. Beard, 1983. Embryo culture of *Helianthus* hybrids. Crop Sci., 23: 1004-1007.

Chandler, J.M. and C.C. Jan, 1985. Comparison of germination techniques for wild *Helianthus* seeds. Crop Sci., 25: 356-358.

Schneiter, A.A. and J.F. Miller, 1981. Description of sunflower growth stages. Crop Sci., 21: 901-903.

Zollinger, R.K., 1997. 1997 North Dakota Weed Control Guide. NDSU Extension Service Circular W-253 Revised. pp. 1-116.

HERENCIA DE LA RESISTENCIA A IMIDAZOLINONAS EN GIRASOL

RESUMEN

Las malezas de hoja ancha causan perdidas de rendimiento considerables a los productores de girasol en todas las regiones del mundo. La resistencia encontrada en una población de girasol a los herbicidas de la familia imidazolinonas: imazethapyr e imazamox, podría ser de gran utilidad para controlar malezas de hoja ancha. La resistencia a herbicidas fué exitosamente transferida desde plantas de girasol silvestre a una linea endocriada de girasol cultivado, denominada HA 425. El objetivo de esta investigación fué estudiar la herencia de la resistencia al herbicida imazamox en la línea HA 425. El análisis de la segregación de plantas en una población F_2 y cruzamientos de prueba, indicaron que la resistencia estaba controlada por dos genes, un gen mayor con un tipo de acción génica semi-dominante (*Imr1*) y un segundo gen (*Imr2*), con efecto modificador cuando el gen mayor estaba presente. La resistencia a herbicidas en girasol puede ser lograda solamente con completa homocigosis (*Imr1, Imr1, Imr2, Imr2*) de los genes de resistencia, por lo que híbridos completamente resistentes requieren tener los dos factores de resistencia en ambos padres.

HÉRÉDITÉ DE LA RÉSISTANCE À L'HERBICIDE IMIDAZOLINONE CHEZ LE TOURNESOL

RÉSUMÉ

Les plantes adventices à feuilles larges sont la cause d'importantes diminutions du rendement du tournesol dans toutes les parties du monde. La résistance aux herbicides basés sur l'imidazolinone, l'imazethapyr et l'imazamox, confirmée dans une population de tournesol sauvage, pourrait être d'une grande importance dans la lutte contre de nombreuses plantes adventices à feuilles larges. La résistance aux herbicides a été transmise avec succès du tournesol sauvage résistant à la ligne inbred de tournesol cultivé HA 425. Le but de cette expérience était d'établir l'hérédité de la résistance à l'herbicide imazamox dans la ligne HA 425. Les rapports de division dans les plantes F_2 et dans les populations contrôle (cross-test) ont démontré que la résistance se trouve sous le contrôle de deux gènes, un gène principal avec un type semi-dominant d'action génétique (*Imr*1) alors qu'un deuxième gène (*Imr2*) a un rôle modificateur quand le gène principal est présent. La résistance du tournesol ne peut être atteinte qu'en transmettant les deux gènes de résistance (*Imr1, Imr1, Imr2, Imr2*) à l'état homozygote dans une ligne inbred ou hybride. Pour obtenir des hybrides tout à fait résistants, il est nécessaire que les deux parents possèdent les facteurs de résistance.